Blood lead levels in Bald Eagles (*Haliaeetus leucocephalus)* in Southeast Alaska by gender and capture location.

Jessica Hambleton

Abstract

Research into the effects of lead on bald eagles has demonstrated that lead levels are higher in bald eagles than other birds at lower trophic levels (Burger and Gochfield 2009). Bald eagle survey data collected in Alaska also tells us that the breeding populations of Alaska contain a substantial proportion of the total number of eagles in North America (Hodges 2011). This project focused on the blood lead levels in previously tagged birds for which blood had been collected by the United States Fish and Wildlife Service. The samples included 32 individuals, with 17 males and 15 females. Males had a mean blood lead level of 0.0283±.0072 mg/L and females had a mean blood lead level of 0.0862 ±0.0866 mg/L (see figure 1). Statistical tests of blood lead level and gender, distance to Greens Creek Mine and distance to Juneau, Alaska all showed non-significance. Future studies focusing on individuals with known breeding areas as well as age class should be conducted.

Introduction

Haliaeetus leucocephalus, or the bald eagle, suffered major population declines until the Bald Eagle Protection Act was put in place in 1940 with the advent of Alaska statehood (Hodges, 2011). While bald eagle populations are recovering or have recovered in many areas, human activities, both intended and unintended, still may have substantial impacts on their populations. In Alaska, specifically Southeast Alaska, the bald eagle is a common inhabitant (Sidle et al, 1986). Living in areas sometimes only accessibly by plane or ferry, problems from human populations may seem as if they would not affect wildlife in these areas, however many aspects of development can have an impact on organisms that live and breed in these areas. As development and human populations in Alaska increase, the impacts on bald eagle populations could change, causing a recurrence of previous population declines.

Lead is a metal pollutant that occurs in streams, coastal waters, and soil. While new evidence suggests the possibility of biomagnification from one trophic level to another of lead in some organisms (Rubio-Franchini and Rico-Martinez 2011), the bioconcentration of lead from the environment to organisms is better known (Gray 2002, Rico 2006). Human use of lead often includes waste incineration, hunting activities, and mining (Rico, 2006). Greens Creek Mine on Admiralty Island is located a mere 93 miles from Haines, Alaska, which sits near the Chilkat River, home to a large seasonal concentration of eagles (more than 3500 eagles) at peak productivity (Sidle et al. 1986). The mine produces lead, amongst other metals, including gold and silver (Hecla Mining Company 2014). Other common sources of lead in Southeast Alaska include large game hunting, fuel consumption, and general products of human activity (Rico 2006, Bedrosian 2012). With a large population and many breeding pairs of bald eagles in Southeast Alaska, it is important to know what impacts human expansion in Southeast Alaska could have on these populations. As an important indicator species, lead contamination in Bald Eagles could indicate further problems in the environment or other trophic levels that have yet to be discovered.

Research into the effects of lead on bald eagles has demonstrated that lead levels have been found to be higher in bald eagles than other birds at lower trophic levels (Burger and Gochfield 2009). Bald eagle survey data collected in Alaska also tells us that the breeding populations of Alaska contain a substantial proportion of eagles in North America (Hodges 2011). With such a large breeding population in close proximity to residential, commercial, and industrial human activity, it is important to study the possibility of lead poisoning in these animals. This project attempted to collect data on the blood lead levels in this specific population. Location, sex, and mercury levels on some birds in Southeast Alaska have been collected, but lead analysis on blood samples of these same birds has not been conducted as of yet.

Methods

Blood samples of bald eagles were provided by Steve Lewis of the United States Fish and Wildlife Service. Samples were heparinized whole blood, frozen at approximately -15 degrees Celsius or colder. Samples were labeled with the banding ID number used for a previous banding project, capture location and dates. Since the samples had been frozen, concern was expressed over the quality of the samples due to this process. Consultation with veterinarians at Southeast Alaska Animal Medical Center, an online query to the Veterinary Information Network, and other sources confirmed that no lead loss occurred when freezing blood samples for lead testing (Wang and Peter, 1985).

These samples were transported to Southeast Alaska Animal Medical Center by the author, and refrigerated overnight at approximately 5.8 degrees Celsius. They were then brought to room temperature at 21.1-23.9 degrees Celsius and placed on a tube rocker. Pre-labeled red top tubes with no additive and no plastic rings inside were used to store 300 µl of each sample. A pre-calibrated Idexx® 300 µl pipette with individual plastic tips for each sample was used to transfer the sample from the original tube to the red top tube.

The original tubes containing the remaining blood were placed back in the freezer at -15 degrees Celsius at Southeast Alaska Animal Medical Center. Three samples from band numbers 816, 848, and 842 were sent out as a preliminary test, while the rest were placed in the freezer along with the original samples. Parafilm® was used to wrap the top of the red top tubes, and these were sent to Phoenix Central Laboratories in Mukilteo, Washington.

Initial attempts with the lead testing on these samples proved difficult, and the samples were returned along with the diluent required for the testing. The author then used a pre-calibrated Idexx® 50 µl pipette to transfer 50 µl of the sample to the diluent according to the directions for the lead testing kit (Magellan Diagnostics, 2012). These were returned to Phoenix Central Laboratories to complete the procedure for lead testing.

Upon receipt of results for the initial samples, the remaining samples were packaged in diluent using the same procedure, and sent to Phoenix Central Laboratories for testing.

Statistical testing was done with SPSS® Statistics version 22. A Shapiro-Wilk test for normality was completed as well as a Spearman rank correlation for both the distance to Juneau, Alaska and Greens Creek Mine from capture location coordinates against blood lead level. The distance for these was found using http://boulter.com/gps/distance entering the coordinates provided for each capture location from the United States Geological Survey Bird Banding Lab, using 58.080964, -134.645117 for Greens Creek Mine and 58.353404, -134.504387 for Juneau, Alaska.

Figure 1. Diluent vials prepared prior to send-out.

Results

The blood lead levels of 32 samples with 17 females and 15 males had a high of 0.255 mg/L and a low of less than 0.014 mg/L. Males had a mean blood lead level of 0.0283±.0072 mg/L and females had a mean blood lead level of 0.0862 ±0.0866 mg/L (see figure 1). Reference ranges were according to Stauber et al 2010, with background at less than 0.2 mg/L, subclinical between 0.2 and 0.5 mg/L, clinical between 0.51 and 1.00 mg/L and lead poisoning above 1.00 mg/L. Results for 5 individuals with less than 0.014 were not included in statistical analysis. A Mann –Whitney U test (p=0.430) determined that the difference in blood lead levels between males and females was non –significant, and a Shapiro-Wilk test (p=0.000 for males and p=0.001 for females) determined that the data were from a non-normal distribution.

With respect to two major potential lead sources—the Hecla Greens Creek Mine on Admiralty Island, and the human population center of Juneau—the blood lead levels when compared to the distance from the capture location coordinates of each individual to these sites was found to be non-significant. A Spearman's rank test (r_s=0.122, p=0.544, n=27, df=25, see figure 2) for the distance from capture site to Green's Creek Mine and the distance from capture site to Juneau, Alaska (r_s=0.085, p=0.73, n=27, df=25, see figure 3) found that the results for both were non-significant.

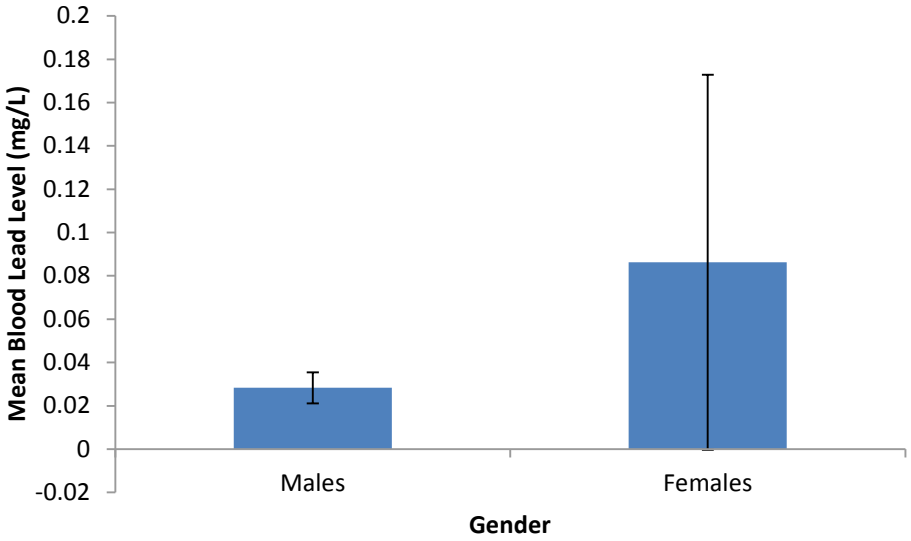

Figure 2. Graph displaying the mean blood lead levels in mg/L for males and females sampled with error bars representing the relative standard deviation.

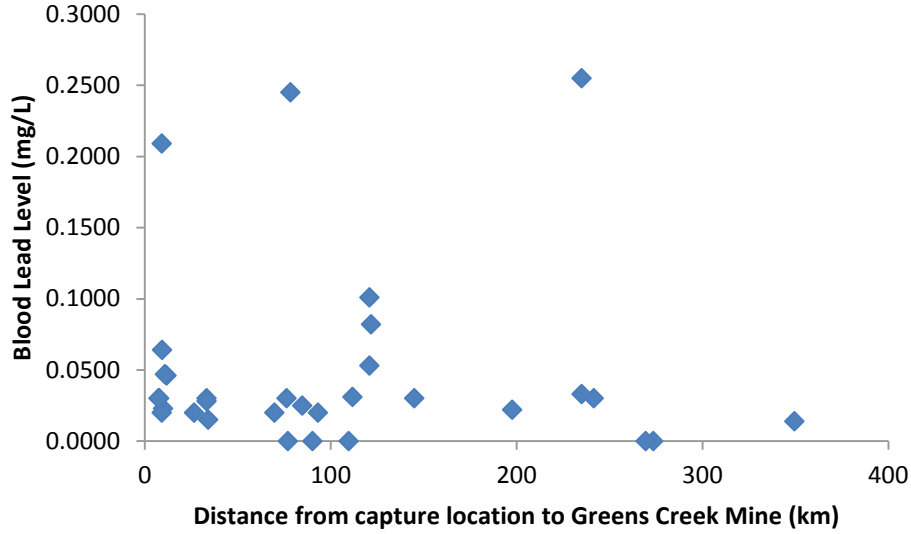

Figure 3. Distance from capture location to Greens Creek Mine (in kilometers) versus blood lead level (mg/L).

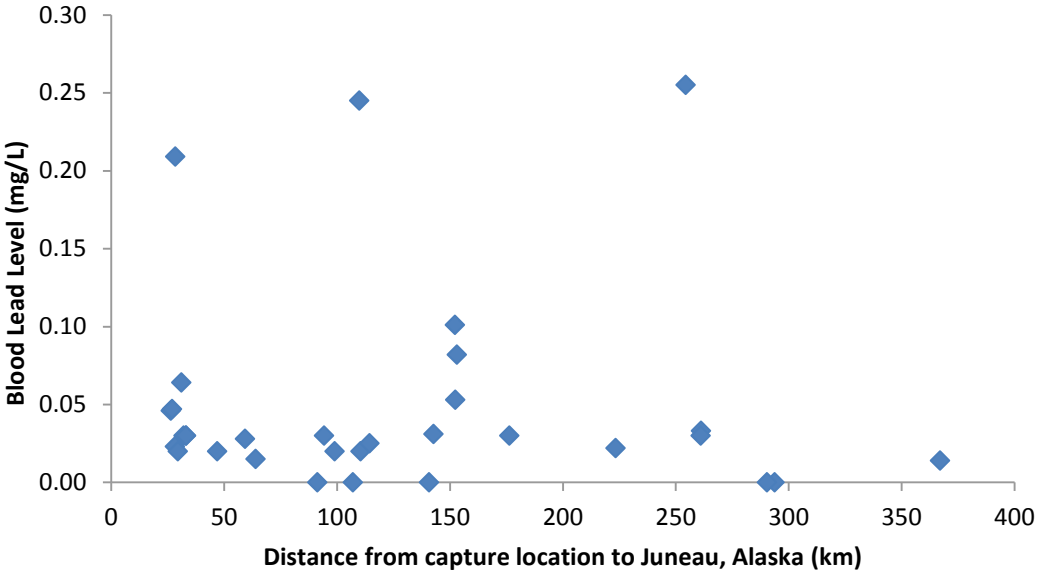

Figure 4. Distance from capture location to Juneau, Alaska in kilometers versus blood lead level (mg/L)

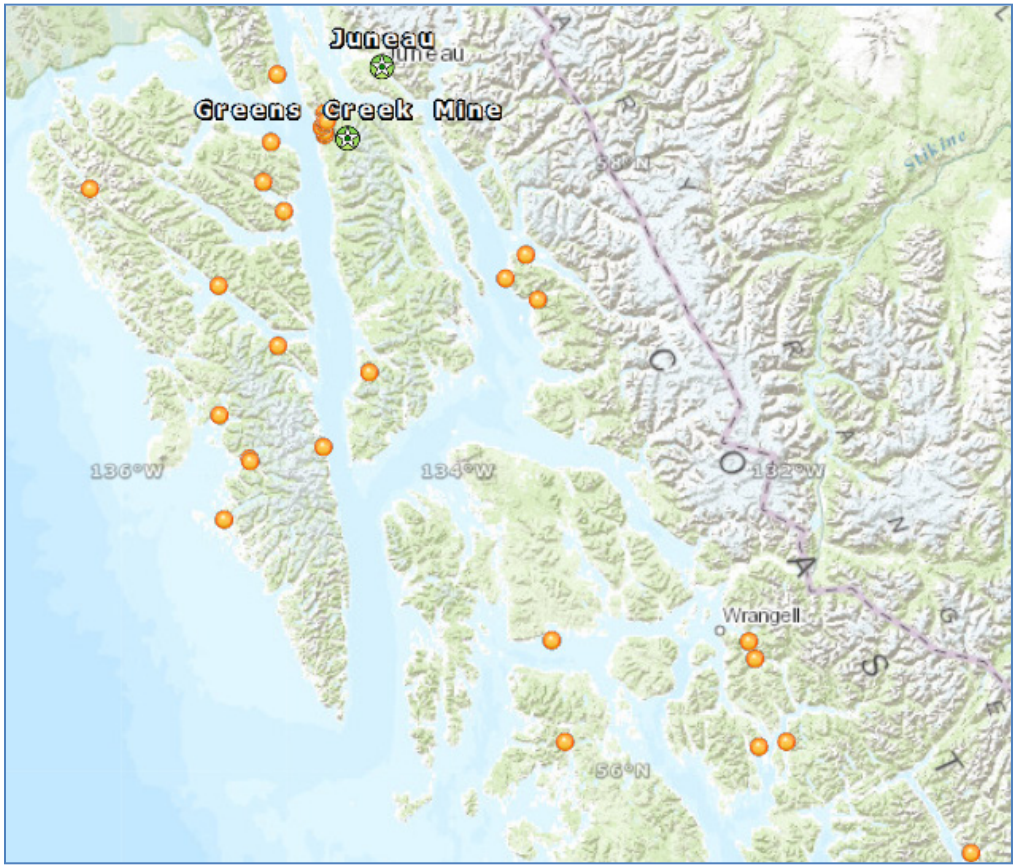

Figure 5. Map of GPS coordinates of capture locations for 32 individuals for which blood lead levels were tested, with relative locations of Greens Creek Mine, and Juneau Alaska. (Created in ArcGIS)

Discussion

No significant difference was found between the blood lead levels of females and males. This is consistent with the findings of Burger and Gochfield (2009) and Bedrosian et al. (2012). However, it is still worth noting that the mean blood lead level was higher in females, and that the three blood lead levels that were not classified as background were female. The relative normality of the blood lead levels indicates that either the individuals sampled had not spent enough time near lead contamination for it to bioaccumulate to levels considered toxic, or that the lead levels they were exposed to were minimal (Stauber et al, 2010; Gray, 2002). Either scenario presents optimistic results that could indicate a relatively healthier population than would be expected considering the increase in human population and development in Southeast Alaska. We know that lead is found in higher concentrations in *H. leucocephalus,* as a higher trophic level bird (Burger and Gochfield, 2009), and as an important indicator species, we can hypothesize on the relative lead levels of other species.

The potential exists for blood lead levels to increase in the population of Southeast Alaskan *H. leucocephalus.* Since these individuals have the potential to travel vast distances during migrations, it is valuable to understand their movements in relation to the bioaccumulation of lead and other toxicants (Bedrosian et al, 2012; Gray 2002).

The distance of capture location in relation to two major lead sources, Greens Creek Mine on Admiralty Island, and Juneau, Alaska also showed no significance. However, the capture location is simply the GPS coordinates of the location where the individual was captured and tagged. Individuals may have been non-breeding, or non-local. This means that individuals may not have spent enough time near these sources to have accumulated lead in their bodies (Bedrosian et al 2012).

Other studies have looked at the lead levels of *H. leucocephalus* scavenging on carcasses contaminated with lead shot during hunting seasons (Stauber et al, 2010; Bedrosian et al, 2012) and found that there is a significant increase in blood lead levels. The date of sampling of the individuals in this study in comparison to the blood lead levels would be an interesting aspect for further study to find whether the blood lead levels of wild Alaskan *H. leucocephalus* would match those of previously studies.

Further research should investigate the contaminant sources for breeding individuals known to spend disproportionate amounts of time in areas of toxicant contamination or lacking contamination. Others have found no significant difference among age class or gender in relation to blood lead levels (Bedrosian et al, 2012; Burger and Gochfield 2009), which is consistent with these results, although due to the lack of samples of various age classes, the variation in lead between these groups could not be tested. Future studies might be able to provide more information should samples from other age classes be obtained.

Lastly, while sources indicate that lead loss does not occur with freezing, testing fresh samples with the same procedure would be a control, and according to the testing kit, better results would be provided with fresh samples (Wang and Peter, 1985; Magellan Diagnostics, 2012). Also, for diagnostic consistency, only samples of whole heparinized blood were used, though some samples in EDTA were available, and the procedure indicated that this was an acceptable alternative, as well as storage in capillary tubes (Magellan Diagnostics, 2012). A study determining the efficacy of these various types of blood storage would provide valuable information regarding blood storage for wildlife, as collecting samples from migratory individuals is significantly more difficult than doing so for domesticated animals and pets.

Acknowledgments

Funding for this project was provided by the Bald Eagle Fund of University of Southeast Alaska. Considerable thanks should be given to Steve Lewis, of the United States Fish and Wildlife Service, for his guidance, and providing the samples that made this project possible. Also, thanks to Heidi Pearson, as a mentor and an advisor, and the staff at Southeast Alaska Animal Medical Center for their patience and assistance. Lastly, to Shannon Pedersen at Phoenix Laboratories for her help in this project.

Literature Cited

Bedrosian B, Craighead D, Crandall R (2012) Lead exposure in bald eagles from big game hunting, the continental implications and successful mitigation efforts. PLoS One 7:e51978. doi: 10.1371/journal.pone.0051978

Burger J, Gochfield M (2009) Comparison of arsenic, cadmium, chromium, lead, maganese, mercury and selenium in feathers in bald eagle (*Haeliaeetus leucocephalus*), and comparison with common eider (*Somateria mollissima*), glaucous-winged gull (*Larus glaucescens*), pigeon guillemot (*Cepp*). Env Mont Assess 152:357–367.

Elliott KH, Elliott JE, Wilson LK, et al. (2011) Density-Dependence in the Survival and Reproduction of Bald Eagles: Linkages to Chum Salmon. J Wildl Manage 75:1688–1699. doi: 10.1002/jwmg.233

Gray JS (2002) Biomagnification in marine systems: the perspective of an ecologist. Mar Pollut Bull 45:46–52.

Hodges Jr. J (2011) Bald Eagle Population Surveys of the North Pacific Ocean, 1967-2010. Northwest Nat 92:7–12.

Magellan Diagnostics (2012) LEADCARE Blood Lead Test Kit. 1–2.

Rico P (2006) Toxicology Profile for Lead. Agency Toxic Subst Dis Regist Toxicol Profiles 301–381.

Rubio-Franchini I, Rico-Martínez R (2011) Evidence of lead biomagnification in invertebrate predators from laboratory and field experiments. Environ Pollut 159:1831–5. doi: 10.1016/j.envpol.2011.03.021

Sidle W, Suring L, Hodges Jr. J (1986) Wildlife and Fisheries Habitat Management Notes: The Bald Eagle in Southeast Alaska. Juneau

Stauber E, Finch N, Talcott PA, Gay JM (2010) Lead Poisoning of Bald (*Haliaeetus leucocephalus*) and Golden (*Aquila chrysaetos*) Eagles in the US Inland Pacific Northwest Region--An 18-year Retrospective Study: 1991-2008. J Avian Med Surg 24:279–287.

Wang S, Peter F (1985) The stability of human blood lead in storage. J Anal Toxicol 9:85–88.

(2014) Hecla Mining Company--Greens Creek. http://www.hecla-mining.com/operations/operations_greenscreek.php. Accessed 27 Jan 2014

(2010) Phoenix Central Laboratories. http://www.pclv.net/.